Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/CA05/000085

International filing date: 26 January 2005 (26.01.2005)

Document type: Certified copy of priority document

Document details: Country/Office: CA
Number: 2,456,294
Filing date: 26 January 2004 (26.01.2004)

Date of receipt at the International Bureau: 02 March 2005 (02.03.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

PCT/CA2005/000085
15 FEBRUARY 2005 15.02.05

Office de la propriété intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An Agency of Industry Canada

Bureau canadien des brevets
Certification

Canadian Patent Office
Certification

La présente atteste que les documents ci-joints, dont la liste figure ci-dessous, sont des copies authentiques des documents déposés au Bureau des brevets.

This is to certify that the documents attached hereto and identified below are true copies of the documents on file in the Patent Office.

Specification and Drawing, as originally filed, with Application for Patent Serial No: **2,456,294**, on January 26, 2004, by **CANGENE CORPORATION**, assignee of Maurice Genereux, Jitendra Dixit and Reynaldo Angelo C. De Castro, for "Treatment of Dengue Hemorrhagic Fever".

Agent certificateur/Certifying Officer

February 15, 2005
Date

Canada

(CIPO 68)
31-03-04

OPIC CIPO

ABSTRACT

Dengue hemorrhagic fever is treated with Rh antibodies, resulting in an immediate increase in platelets.

TREATMENT OF DENGUE HEMORRHAGIC FEVER

BACKGROUND OF THE INVENTION

Dengue illness is currently the most important mosquito borne viral disease in the tropical areas of the world. It has been estimated that 50 million cases of dengue occur annually worldwide. Approximately 500,000 of such cases develop into the more severe dengue hemorrhagic fever (DHF), which requires hospitalization and has a case fatality rate varying from 1 to 5%. A significant number of deaths, around 24,000, occur each year, most of them in children (WHO Fact Sheet No 117, Revised April 2002). Dengue and DHF are caused by one of four closely related, but antigenically distinct, dengue virus serotypes (DEN-1, DEN-2, DEN-3 and DEN-4), of the genus *flavivirus*. Recovery from infection by one provides lifelong immunity against that serotype but confers only partial and transient protection against subsequent infection by the other three serotypes. There is evidence that subsequent infections increase the risk of the more serious disease resulting in DHF.

Classic dengue fever is characterized by acute onset of high fever, frontal headache, retro-orbital pain, myalgias, arthralgias, nausea, vomiting, and often a maculopapular rash. In addition, many patients may notice a change in taste sensation. Symptoms tend to be milder in children that in adults. The disease manifestations can range in intensity from unapparent illness to the symptoms described above. The acute phase of up to 1 week is followed by a 1- to 2-week period of convalescence, which is characterized by weakness, malaise, and anorexia.

During the first few days of illness, dengue hemorrhagic fever (DHF), a severe and sometimes fatal form of dengue, may resemble classic dengue or other viral symptoms. Patients with DHF may have fever lasting 2 to 7 days and a variety of non-specific signs and symptoms. At about the time the fever begins to subside, the patient may become restless or lethargic, show signs of circulatory failure, and experience hemorrhagic manifestations. The most common of these manifestations are skin hemorrhages such as petechiae, purpura, or ecchymoses, but may also include epistaxis, bleeding gums, hematemesis, and melena. The condition of DHF

patients may rapidly evolve into dengue shock syndrome (DSS), which, if not immediately corrected, can lead to profound shock and death. Advance warning signs of DSS include severe abdominal pain, protracted vomiting, marked change in temperature (from fever to hypothermia), or change in mental status (irritability or obtundation). Early signs of DSS include restlessness, cold clammy skin, rapid weak pulse, and narrowing of pulse pressure and/or hypotension.

The major pathophysiologic hallmarks of DHF/DSS are plasma leakage due to increased vascular permeability and abnormal hemostasis. Hypovolemic shock occurs as a consequence of, and subsequent to, critical plasma volume loss. Abnormal hemostasis includes increased capillary fragility, thrombocytopenia, impaired platelet function, and consumptive coagulopathy. In the most severe form, disseminated intravascular coagulopathy (DIC) contributes to varying degrees of bleeding (Malasit,P, Surface Associated Complement Fragment on Platelets of Dengue Patients, SEA J TM 1990).

Thrombocytopenia is common in dengue fever and a constant finding in DHF/DSS. The platelet count usually drops to below 100,000 1-2 days before defervesce and remains low for 3-5 days in most cases. The levels then increase rapidly to normal during convalescence. DHF/DSS induced thrombocytopenia has been suggested to be caused by both dengue virus-induced bone marrow suppression of platelet production (La Russa VF, Innis BL, Baillieres Clin Haematol 1995) and antibody-mediated destruction of platelets by complement activation, (Mitrakul, C, Bleeding Problems in DHF, SEA J TM 1987) a mechanism similar to the pathophysiology of childhood idiopathic thrombocytopenic purpura (ITP). As such, the conventional therapy for thrombocytopenia associated with DHF/DSS includes intravenous replacement of fluids and electrolytes, plasma or colloids like albumin, dextran, gelatin and platelet transfusion. For severe DSS, steroids have been used such as the prednisolone derivative methylprednisolone [(6 alpha,11 beta)-11,17,21-trihydroxy-6-methylpregna-1,4-diene-3,2-dione]. With the current therapy, fatality rates among those for DHF range from 1-5% and fatality rates among those with DSS may be as high as 44%. DHF/DSS can occur in children and adults.

Immunoglobulins have been used to treat thrombocytopenia of immune and non-immune origin such as HIV related thrombocytopenia as well as thrombocytopenia associated with infectious mononucleosis. Results of most studies show favorable results. In 1991, differences were noted between clinical effects of IVIG and IV anti D especially in HIV with the latter showing better results (Scarandavao, A., IV anti D in Treatment of ITP, Blood. Vol 89, No.8 1977 pp2689-2700). However, in the treatment of HIV, the thrombocytopenia is the result of a chronic infection, and is not an immediate threat to the life of the patient. In contrast to HIV associated thrombocytopenia, dengue thrombocytopenia is acute and the cause of death associated with DFH/DSS. In 1999 Frias used IVIG in patients with DHF and reported a decrease in the mortality rates of patients who received IVIG (Frias, M., Use of IVIG in DSS, Double Blind Placebo Controlled Trial, June 1999).

Rh(D) immunoglobulin has been used to treat HIV-associated ITP in adults and children (Gringeri, A., et al., Br. J. Haematol. 1992 80(3): 337-40; Rossi, E., et al., Haematologica 1991 76(2): 141-9; Landonio, G., et al., AIDS 1990 4(1): 29-34; Brusamolino, E., et al., Haemotologica 1989 74(1): 51-6; Cattaneo, M., et al., Blood 1989 73(1): 357); and Bussel et al., Blood, 1991, 77:1884-1893). The majority of patients treated with Rh(D) immunoglobulin respond with increased platelet counts, but the platelet response is temporary and lasts about 3 weeks. Rh(D) immunoglobulin treatment has been ineffective in Rh negative patients, and in all patients that have received a splenectomy prior to therapy.

SUMMARY OF THE INVENTION

According to a first aspect of the invention, there is provided a method of treating acute thrombocytopenia associated with dengue hemorrhagic fever comprising administering to an individual in need of such treatment an effective amount of Rh antibodies. In an alternate aspect of the invention, the acute thrombocytopenia is characterized by platelet counts below 100,000 per mm^3, below 75,000 per mm^3 or below 60,000 per mm^3.

According to a second aspect of the invention, there is provided a method of

treating acute thrombocytopenia comprising administering to an individual in need of such treatment an effective amount of Rh antibodies, wherein platelets of said individual have dropped below 100,000 per mm^3. In an alternate aspect of the invention, the acute thrombocytopenia is characterized by platelet counts below 100,000 per mm^3, below 75,000 per mm^3 or below 60,000 per mm^3.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows a graph of the mean platelet counts from 18 patients suffering from DFH or DSS before and after treatment with RhD antibodies.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned hereunder are incorporated herein by reference.

As used herein, "Rh antibodies" are understood to include antibodies specific for antigens of the Rh blood group system, or epitopes thereof. (See The Rh Blood Group System, in Blood Transfusion in Clinical Medicine., ed. Mollison PL et al., chapter 8, page 328, for a review of the Rh blood group antigens, which is incorporated in its entirety herein by reference). Examples of Rh antibodies include anti-D (also known as anti-Rh_o, and also referred to herein as anti-Rh_o(D)); anti-C (also known as anti-rh'); anti-E (also known as anti-rh"); anti-c (also known as anti-hr'); and, anti-e (also known as anti-hr").

As used herein, the term "treating" in its various grammatical forms refers to preventing, curing, reversing, attenuating, alleviating, minimizing, suppressing or halting the deleterious effects of a disease state, disease progression, disease causitive agent or other abnormal condition.

As used herein, "effective amount" refers to the administration of an amount of

a given compound that achieves the desired effect.

Described herein are methods of treating dengue hemorrhagic fever with Rh antibodies. As discussed below and in the accompanying figure, the treatment results in an immediate increase in platelets.

The methods, compositions and uses of the invention, use Rh antibodies. Within the context of the present invention, Rh antibodies are understood to include antibodies specific for antigens of the Rh blood group system, or epitopes thereof. (See The Rh Blood Group System, in Blood Transfusion in Clinical Medicine., ed. Mollison PL et al., chapter 8, page 328, for a review of the Rh blood group antigens, which is incorporated in its entirety herein by reference). Examples of Rh antibodies include anti-D (also known as anti-Rh_o, and also referred to herein as anti-Rh_o(D)); anti-C (also known as anti-rh'); anti-E (also known as anti-rh"); anti-c (also known as anti-hr'); and, anti-e (also known as anti-hr").

As used herein, "dengue hemorrhagic fever" or DHF and "dengue shock syndrome" or DSS are used interchangeably, as both refer to severe outcomes associated with dengue virus infection. It is of note that as used herein these terms also refer to severe thrombocytopenia caused by other means, for example, non-dengue viruses or other microorganisms.

As discussed in the accompanying figures and tables, 18 children suffering from dengue fever were treated with Rh antibodies. On the days immediately prior to treatment, there was a steady decrease in platelet counts. During the normal course of the disease, even with conventional therapy known in the prior art, platelet counts would continue to decrease up to 9 days, during which there is an approximately 1-5% mortality rate. There is no specific treatment or vaccine for dengue fever. However, as discussed above, on treatment with Rh antibodies platelet counts rose immediately.

As will be appreciated by one of skill in the art, the accompanying data clearly indicates that Rh antibodies can be used to treat severe or acute cases of thrombocytopenia, for example, cases of thrombocytopenia wherein platelets have dropped to below 100,000 per mm^3, regardless of the cause of the thrombocytopenia.

Dengue hemorrhagic fever is associated with dengue virus infections, particularly those occurring against a background of previous exposure to a dengue virus of another serotype. As will be appreciated by one of skill in the art, this previous exposure may be from a previous infection or may be the result of hyperdermic transmission. Specifically, it is believed that on infection, two types of dengue antibodies are produced, neutralizing antibodies and non-neutralizing antibodies. The neutralizing antibodies bind to the dengue virus and prevent the virus from further infecting cells. It is believed that non-neutralizing antibodies trigger a pathogenic cascade when such antibodies bind to the virion's surface and promote absorption of secondary dengue viruses on the target cell, thereby enhancing infection. Thrombocytopenia associated with DHF/DSS is believed to result from such non-neutralizing antibodies binding to either platelets or dengue viral antigens in infected platelets, which results in rapid clearance of such opsonized platelets from the circulation.

The prior art teaches treating dengue hemorrhagic fever with purified Ig administered intravenously (IVIG). By definition, an IVIG preparation will contain antibodies for a variety of antigens, but will not have antibodies against antigens endogenous to humans. It is believed that administration of IVIG to a patient results in a down regulation in the production of endogenous antibodies, thus alleviating the thrombocytopenia associated with DHF/DSS.

While not wishing to be bound to a specific theory, it is believed that treatment of an Rh positive DHF/DSS patient with Rh antibodies results in red blood cell opsinization. Clearance of the opsinized red blood cells induces Fc receptor blockage in the reticuloendothelial system, which is seen primarily as an action on the splenic macrophages and other monocytes/macrophages. The Fc receptor blockage in turn reduces peripheral consumption and destruction of platelets opsinized with non-neutralizing dengue antibodies.

As discussed above, Rh antibodies were shown to temporarily increase platelet counts in individuals suffering from HIV-associated ITP. However, in HIV patients, the thrombocytomenia is chronic, rarely life threatening with platelet levels rarely dropping

below 100,000 mm^3, and the thrombocytopenia returns after 3 weeks. Given the transient nature of the increase in platelets seen in HIV patients, it is surprising that Rh antibodies would be so effective in treating dengue patients with acute thrombocytopenia, specifically, DHF/DSS patients wherein the thrombocytopenia is acute, has a rapid onset and is life threatening.

As will be apparent to one of skill in the art from the accompanying data and observations, Rh antibodies have several advantages over treatment with IVIG. Specifically, the peak platelet count following treatment with Rh antibodies is higher than with IVIG and the cost of Rh antibodies is significantly lower compared to IVIG. Furthermore, the side effects with Rh antibodies are less severe compared to IVIG and the response rate, characterized by >25,000 per µl, is higher with Rh antibodies (82%) than IVIG (48%).

Generally Rh antibodies for use in the invention are selected depending on the Rh antigens present/absent on the red cells of the subject to be treated. Anti-Rh_o(D) is preferably used to treat Rh positive (i.e. D positive) subjects, and anti-c is preferably used to treat Rh negative (i.e. D negative) subjects.

The Rh antibodies used in the present invention may be preparations from plasma enriched for Rh antibodies, monoclonal antibodies, polyclonal antibodies, antibody fragments (e.g., Fab, and F(ab')2), and recombinantly produced binding partners.

Preparations with a high Rh antibody content may be isolated as an immunoglobulin fraction from plasma, preferably human plasma, using conventional techniques. For example, an Rh IgG fraction may be isolated from human plasma by (a) the Cohn cold ethanol method and modifications thereto (e.g. see Barandun, S., et al., 1962, Vox Sang. 7, 157-174); (b) the DEAE-Sephadex method described by Hoppe, H. H. et al., 1967, Munch. Med. Wochenschr. 109:1749-1752); or (c) the anion-exchange method described in Canadian Patent No. 1,201,063, and modifications thereto. Commercially available Rh immunoglobulin preparations may also be used in the methods, uses and compositions of the invention. For example, anti-Rh_o(D) preparations including WinRho®., WinRho SD®, WinRho SDF®

(Cangene Corporation, Winnipeg, Canada), RHOGAM Rh_o(D) Immune Globulin (Johnson & Johnson, Piscataway, New Jersey, USA) and HypRho®-D (Miles Canada Inc., Etobicoke, Canada) may be used in the present invention.

In an embodiment of the invention an Rh_o(D) IgG fraction is prepared by contacting an aqueous animal plasma fraction (preferably human plasma) containing IgG with one or more chromatographic separation columns to produce a purified IgG rich fraction. The aqueous animal plasma fraction used in the process may be normal non-immunized plasma from an animal source, preferably a human source, or hyper-immune plasma such as plasma from Rh alloimmunized donors. The plasma is modified to the ionic strength and pH of the initial buffer used with the chromatographic separation column. In an embodiment of the invention, the aqueous animal plasma fraction is contacted with one or more, preferably one or two anionic exchangers to produce a purified IgG rich fraction.

The purified Rh antibody rich fraction may optionally be treated with a solvent and detergent to inactivate lipid envelope viruses. Suitable solvents and detergents which may be used to inactivate lipid envelope viruses include Triton X-100 and tri (n-butyl) phosphate. The solvents and detergent may be removed by conventional methods such as reverse phase chromatography.

The chromatographic separations may be carried out on anion exchangers using the procedure as described in Canadian Patent No. 1,201,063 which is incorporated herein in its entirety by reference. By way of example, the aqueous animal plasma fraction is applied to an anion exchange column which may contain an agarose cross-linked anionic exchange resin such as DEAE-Sepharose CL6B or DEAE-Biogel, and an IgG rich fraction is obtained by elution with an equilibrating buffer. The IgG rich fraction may be concentrated for example by ultrafiltration. The IgG rich fraction is applied to a second different anion exchange column such as DEAE-Biogel™, or Deae-Sephadex™ A-50. A purified IgG rich fraction is isolated by elution with an appropriate equilibrating buffer.

The purified Rh antibody fraction may be formulated with a wetting agent such as Polysorbate 80, also known as Tween 80. The wetting agent reduces the amount

of fragmentation over extended periods of time to provide a highly stable preparation enriched for Rh_o(D) antibodies. The purified IgG fraction may be further purified using ultrafiltration.

The purified Rh antibody containing IgG rich fraction obtained using the process described above may be further stabilized by the addition of stabilizers such as mannitol, glycine (e.g. 0.1 M glycine), and sodium chloride (e.g. 0.15 M sodium chloride), and the pH of the fraction may be adjusted within the range 4.0 to 5.4. The resulting preparation may be sterilized for example, by filtration, and it may be used in this form. If desired the preparation may be freeze-dried, and reconstituted using a suitable solution, e.g. 0.9% sodium chloride, or phosphate buffered saline.

A preferred preparation obtained using the process described above has the following characteristics: 2-3% human immune globulin, no or very low level buffer, essentially no ionic strength, 10 ppm polysorbate 80, 10% sorbitol, pH 4.0.

Rh antibodies used in the present invention may be preparations containing polyclonal antibodies specific for Rh antibodies generated using conventional procedures in humans and animals. By way of example, the Rh_o(D) antigen is used to immunize the animal through intraperitoneal, intramuscular, intraocular, or subcutaneous injections, with or without an adjuvant such as Freund's complete or incomplete adjuvant. Following several booster immunizations, samples of serum are collected and tested for reactivity to the antigen in standard assays, examples of which are described below. Particularly preferred polyclonal antisera will give a signal on one of the assays that is at least three times greater than background. Once the titer of the animal has reached a plateau in terms of its reactivity to the antigen, larger quantities of antisera may be readily obtained either by periodic (e.g. weekly) bleedings, or by exsanguinating the animal.

Human Rh antibodies may also be produced in human volunteers. For example, an anti-Rh_o(D) preparation may be obtained from a subject initially immunized naturally during an Rh incompatible pregnancy, and given booster immunizations of whole Rh positive red cells.

Monoclonal Rh antibodies may also be readily generated using conventional

techniques (see U.S. Pat. Nos. RE 32,011, 4,902,614, 4,543,439, and 4,411,993 which are incorporated herein by reference; see also Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses, Plenum Press, Kennett, McKearn, and Bechtol (eds.), 1980, and Antibodies: A Laboratory Manual, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988, which are also incorporated herein by reference).

Other techniques may also be utilized to construct monoclonal antibodies (see William D. Huse et al., "Generation of a Large Combinational Library of the Immunoglobulin Repertoire in Phage Lambda," Science 246:1275-1281, December 1989; see also L. Sastry et al., "Cloning of the Immunological Repertoire in Escherichia coli for Generation of Monoclonal Catalytic Antibodies: Construction of a Heavy Chain Variable Region-Specific cDNA Library," Proc Natl. Acad. Sci USA 86:5728-5732, August 1989; see also Michelle Alting-Mees et al., "Monoclonal Antibody Expression Libraries: A Rapid Alternative to Hybridomas," Strategies in Molecular Biology 3:1-9, Jan. 1990; these references describe a commercial system available from Stratacyte, La Jolla, Calif., which enables the production of antibodies through recombinant techniques). Similarly, binding partners may also be constructed utilizing recombinant DNA techniques to incorporate the variable regions of a gene which encodes a specifically binding antibody (See Bird et al., Science 242:423-426, 1988).

Monoclonal Rh_o(D) antibodies and methods for preparing same which may be used in the present invention are described in WO 91/07492 Canadian Patent Nos. 1,303,533 and 1,303,534, EP 251440 (all to the Central Blood Laboratory Authority); WO 94/00561 (National Reg. Association Transfusion Sanguine/Biotest Pharma GmBH); WO 91/05800 (Foundation Centre National Transfusion Sanguine); EP 523949 (Welcome Foundation), DD 338332 (Humboldt University, Berlin); GB 2127434 (University of London); JP 60115530 (Wako Pure Chem.); and, SU 1678830 (Research Institute Hematology).

It will be apparent to one skilled in the art that the preparations used in the present invention may contain more than one type of Rh antibody. For example, a

preparation may contain anti-Rh_o(D) and anti-c.

Compositions/preparations of the invention contain Rh antibodies, either alone or together with other active substances. Such compositions are for intravenous, intranasal, intramuscular, subcutaneous, oral, enteral or parenteral use. In particular, those forms for intramuscular or subcutaneous administration are used, or forms for infusion or intravenous injection are used, which can be prepared as solutions of the antibodies or as powders of the antibodies to be mixed with one or more pharmaceutically acceptable excipients or diluents, suitable for the aforesaid uses and with an osmolarity which is compatible with the physiological fluids. For example, as described herein, an Rh antibody preparation may be formulated with a wetting agent and/or stabilized by addition of a stabilizer. Preferably, the preparations are in a form suitable for intravenous or intramuscular administration (e.g. WinRho SD®, Cangene Corporation, Winnipeg, Canada). When administering the compositions/preparations of the invention by injection, the administration may be by continuous infusion, or by single or multiple boluses.

In an embodiment of the invention, forms for intravenous injection or infusion are selected to maximize drug bioavailability, reduce dosage, and to elicit faster pharmacodynamic action i.e. reticuloendothelial blockage. For example, Rh negative subjects were injected with adult and fetal Rh positive red blood cells and subsequently WinRho™ (e.g. 120 µg) was administered by intravenous or intramuscular injection. Peak plasma levels of WinRho™ were achieved immediately after intravenous injection but were only achieved 24 hours after intramuscular injection. Intravenous injection also produced two-fold higher peak plasma levels than intramuscular injection. Clearance of Rh positive red blood cells was complete within 8 hours of intravenous administration, and within 24 hours of intramuscular administration (Bowman, J. M., et al., CMA Journal 123:1121-1125, 1980).

The compositions/preparations of the invention may contain one or more Rh antibodies together with one or more other active substances. The Rh antibodies may also be administered in combination with human cytokines or human interferon. The Rh antibodies and active substances may be administered by any conventional

means available for the use in conjunction with pharmaceuticals, either as individual separate dosage units administered simultaneously or concurrently, or in a physical combination of each component in a single or combined dosage unit. The antibodies and active substances can be administered alone, but are generally administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice as described herein.

The combination of Rh antibody and active substances may result in a synergistic action which enhances the effects of the Rh antibodies, or enhances the effects of the active substances. The doses of Rh antibodies and active substances may be each selected so that the Rh antibodies and active substances alone would not show a full effect.

The preparations of the invention can be intended for administration to humans or animals. An appropriate preparation may be selected for a particular subject based on the presence/absence of Rh antigens on the surface of the red blood cells of the subject. Generally, Rh positive (i.e. D positive) subjects are treated with anti-Rh_o(D), and Rh negative (i.e. D negative) subjects are treated with anti-c.

Dosages to be administered depend on individual needs, on the desired effect, and on the chosen route of administration. Daily dosages to humans by intramuscular or intravenous injection generally vary between about 10 µg to 400 µg per kg body weight (50 IU to 2000 IU per kg body weight). For intravenous injection or infusion, the preferred dosage is about 10 to 200 µg per kg body weight (50 IU to 1000 IU per kg body weight). Intravenous dosages which are greater than about 20 µg per kg body weight may be more effective in effecting reticuloendothelial blockage. Preferably the dosage for intravenous injection is about 50 to 75 µg per kg body weight (250 IU to 375 IU per kg body weight). The preferred dosage for intramuscular injection is about 20 µg to 400 µg per kg body weight (100 IU to 2000 IU per kg body weight). The lower dosages provided in the present invention reduce the risk of adverse reactions such as cardiovascular/thromboembolic events.

The compositions described herein can be prepared by per se known methods for the preparation of pharmaceutically acceptable compositions which can be

administered to patients, such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985). On this basis, the compositions include, albeit not exclusively, solutions of the Rh antibodies in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

Pharmaceutical techniques may also be employed to control the duration of action of the compositions/preparations of the invention. Control release preparations may be prepared through the use of polymers to complex, encapsulate, or absorb the Rh antibodies.

As discussed above, the Rh antibodies may be used to treat, alleviate or prevent thrombocytopenia associated with dengue fever and/or dengue hemorrhagic fever. As a consequence, treatment with an effective amount of Rh antibodies will accomplish at least one of the following: restore platelets to above 50,000 mm^3, or an increase in platelet count by 20,000 per mm^3 at 48 hours after treatment with Rh antibodies; stablize hematocrit; improve clinical picture; prevent or reverse DSS; promote recovery from shock and reduce risk of hemorrhage.

Having generally described the invention, the same will be more readily understood through reference to the following example which is provided by way of illustration, and it is not intended to be limiting of the present invention.

Example 1

Objectives: To describe the safety and efficacy of anti-D (WinRho) in affecting the outcome on pediatric patients with Dengue Hemorrhagic Fever (DHF)

Outcome Measure: Mortality was the main outcome. Secondary outcomes included rise of platelet count, prevention of shock, duration of shock, and duration of hospital stay.

Study Population Eighteen (18) pediatric patients with clinical diagnosis of Dengue Hemorrhagic Fever (DHF).

Inclusion Criteria

1. Patient aged less than 19 yrs old
2. Clinical diagnosis of DHF based on the WHO criteria and classification.

A. Fever- acute onset, continuous, high, lasting 2-7 days.

B. Hemorrhagic manifestations including at least a (+) tourniquet test and minor or major bleeding phenomena such as petecchiae, purpura, gum bleeding.

C. Thrombocytopenia with platelet count below 100 x 10^9 per dL but above 60 x 10^9 per dL.

D. Hemoconcentration: Hct increased by 20% or more

3. Diagnosis of DHF confirmed by Elisa.
4. Rh positive phenotyping

Exclusion Criteria

1. Hematologic diseases such as leukemias, aplastic anemia, and other disorders of hemostasis.
2. History of significant allergy or atopy to plasma products or other plasma derivatives.
3. Patients who in the course of their present admission received platelet concentrates and steroids.
4. Hemoglobin less than 10 g/dL
5. Clinical diagnosis of DSS as manifest with Hypotension, narrow pulses
6. Rh. Negative phenotype

Screening:

CBC with differential

Rh phenotyping

Platelet count

Elisa confirmation of Dengue Hemorrhagic Fever (Flavivirus)

24 hours post treatment:
CBC with differential
Platelet count

72 hours post treatment:
CBC with differential
Platelet count

7 days post treatment:
CBC with differential
Platelet count

14 days post treatment:
CBC with differential
Platelet count

21 days post treatment:
CBC with differential
Platelet count

Study Drugs and Administration

The patients received a single intravenous dose of commercially available anti-D immunoglobulin (WinRho SDF™) at a dose of 250 IU per Kg administered over 15 minutes. The drug is supplied as 1500 IU per vial.

Outcome Measures

Mortality and Morbidity

Table 1 shows the average numbers of platelets per mm^3 for the 18 DFH/DSS patients. As expected in thrombocytopenia, the number of platelets is lower than normal, and the platelets numbers are decreasing. Typically, the platelet numbers will

decrease for 9 days, at which point the patients will either recuperate, or succumb to the DFH/DSS. At day 0, the patients were administered WinRho SDF™ at a dose of 250 IU per Kg. For all patients, there was an immediate increase in platelet numbers within 24 hours of treatment with WinRho SDF™.

Of the 18 DFH/DSS patients treated with WinRho SDF™, all recovered fully with no mortalities. Additionally, there were no reports of any adverse events among the 18 patients commonly associated with dengue, such as complications referable to the cardiovascular, central nervous, respiratory and renal systems as described by the WHO.

While the preferred embodiments of the invention have been described above, it will be recognized and understood that various modifications may be made therein, and the appended claims are intended to cover all such modifications which may fall within the spirit and scope of the invention.

CLAIMS

1. A method of treating thrombocytopenia associated with dengue hemorrhagic fever comprising administering to an individual in need of such treatment an effective amount of Rh antibodies.

2. A method of treating acute thrombocytopenia comprising administering to an individual in need of such treatment an effective amount of Rh antibodies, wherein platelets of said individual have dropped below 60,000 per mm^3.

DHF AND IVIg - Platelet response

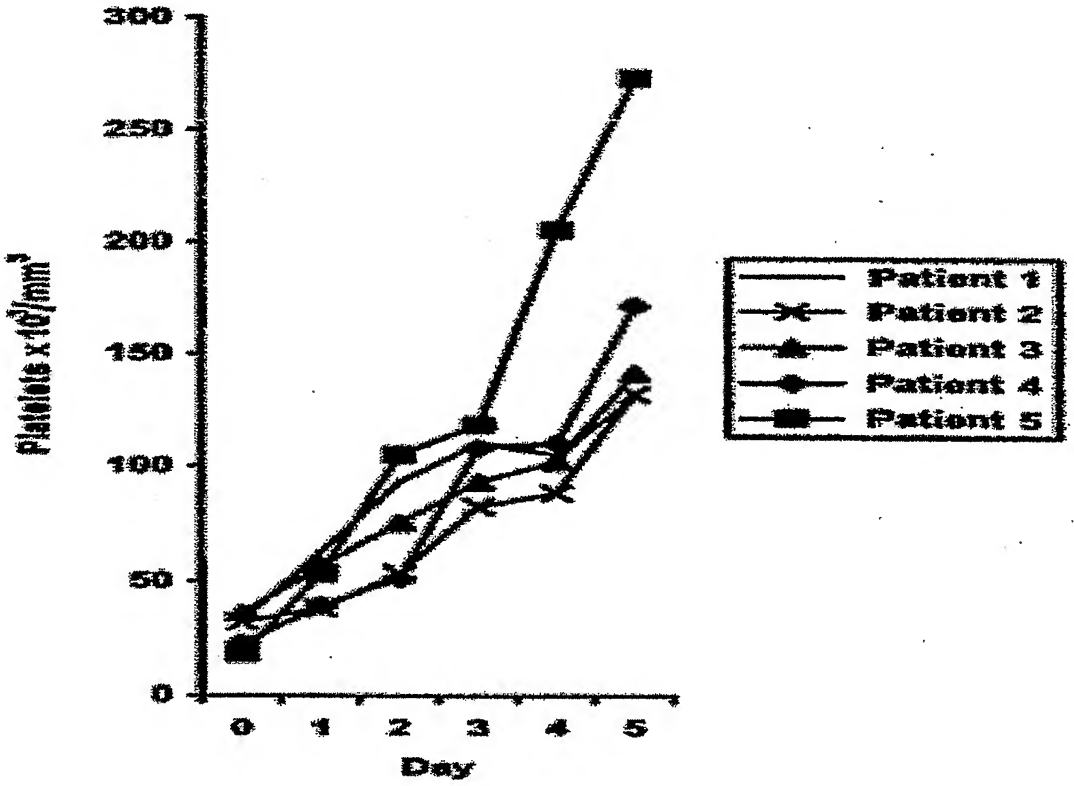

Figure 1. Recovery of platelet counts for 5 patients with dengue hemorrhagic fever treated with high doses of intravenously administered immunoglobulin (IVIG). Day 0, day IVIG therapy initiated.